

The Prognostic and Predictive Role of Histology in Advanced Non-small Cell Lung Cancer

A Literature Review

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Introduction: The importance of non-small cell lung cancer (NSCLC) histologic subtype has increased during the last few decades because of an unprecedented shift in epidemiology and an increasing number of target-specific chemotherapeutic agents. This review examined histology as a potential prognostic and/or predictive factor of clinical outcomes in advanced NSCLC.

Methods: Literature searches of articles from 1982 to 2007 were conducted. We identified publications detailing phase II or III studies, retrospective analyses, and meta-analyses that reported a statistically significant prognostic or predictive role for histology.

Results: Of 408 publications identified, 11 reported a prognostic association between histology and clinical outcomes, and 7 suggested that histologic subtype was predictive of outcomes in patients with advanced NSCLC treated with specific cytotoxic chemotherapy regimens. Fourteen publications reported histology was prognostic and/or predictive in patients treated with epidermal growth factor receptor inhibitors. Inadequate data collection, test methodology, or study design—including insufficient sample size, misclassified samples, and grouping of histologic subtypes for analysis—may have obscured the interpretation of the role of histology in many of the studies.

Conclusions: Although differences in study design and analyses make definitive conclusions difficult, evidence suggests that histology may be prognostic or predictive of clinical efficacy outcomes. To determine which patients would benefit from specific treatments

and to further understand the role of histology, future studies should focus on establishing a definitive histologic diagnosis, and should include an analysis of histologic subtypes and efficacy outcomes.

Key Words: Non-small cell lung cancer, Histology, Chemotherapy, Prognostic, Predictive.

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Background

Slow progress continues to be made in prolonging survival in patients with advanced non-small cell lung cancer (NSCLC). To optimize treatment for the individual patient, published clinical trial results regularly provide information on prognostic factors and factors that may predict drug effect. Historically, histologic subtype has not reliably been shown to have prognostic importance in advanced NSCLC.^{1–3} Although histologic subtypes are often described for patients with NSCLC enrolled in clinical trials, analyses that examine a potential association between histology and efficacy outcomes are often not reported.

However, recently presented data from two randomized, controlled phase III trials of cytotoxic chemotherapy have prompted a renewed interest in the impact of NSCLC histology on efficacy outcomes. A retrospective analysis⁴ of a trial comparing second-line pemetrexed with docetaxel in NSCLC⁵ and a prospective analysis of a trial comparing first-line pemetrexed and cisplatin with gemcitabine and cisplatin in NSCLC⁶ identified a statistically significant treatment-by-histology interaction, with longer overall survival exhibited in patients with nonsquamous carcinoma treated with pemetrexed and shorter survival in patients with squamous cell carcinoma treated with pemetrexed. In addition, a third randomized study comparing two doses of second- and third-line pemetrexed in NSCLC also showed that efficacy varied by histology.⁷

Given these results, we examined if previous trials of agents other than pemetrexed had observed an association between histology and clinical outcomes. We reviewed the literature of the last 25 years and identified studies that reported an association between the histologic subtype of advanced NSCLC, patient prognosis, and/or the efficacy of specific chemotherapeutic agents. This review summarizes those studies, examines them in the context of the literature,

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and discusses possible reasons why histology has not been consistently observed as a prognostic and/or predictive factor. Finally, recommendations are made to further our understanding of the role of histology in the treatment of advanced NSCLC.

Rationale

Additional rationale for examining histologic subtype as a possible prognostic factor or predictor of treatment efficacy in late-stage NSCLC is multifold. First, NSCLC cellular subtypes have different embryologic origins and arise in different anatomic locations (central versus peripheral airways),⁸ and therefore might have differing capacities for drug transport, binding, bioactivation, and metabolism. Additionally, since certain mutations associated with NSCLC occur more frequently in certain cell types (for example, the *ras* proto-oncogene⁹ and epidermal growth factor receptor [EGFR] mutations¹⁰ occur predominantly in lung adenocarcinomas), changes in cell responsiveness associated with those mutations could follow the same pattern.

Likewise, new chemotherapies with selective targets might have a greater impact on one histologic subtype over another given differences in expression of the target among cell types. For example, thymidylate synthase (TS), an enzyme involved in DNA biosynthesis and targeted by antimetabolites (such as 5-fluorouracil and pemetrexed), has higher mRNA and protein expression in squamous cell carcinoma compared with adenocarcinoma.¹¹ High TS expression levels also correlate with poor prognosis in NSCLC.¹² It has been hypothesized that low TS expression might predict better clinical outcome in response to treatment with TS inhibitors such as pemetrexed. Another example is ERCC1 (excision-repair cross complementation group 1), a protein implicated in DNA adduct repair that has higher expression in squamous cell carcinoma.¹³ High ERCC1 gene expression was associated with shorter survival in samples derived from patients with advanced NSCLC.¹⁴ Furthermore, a survival benefit for cisplatin-based adjuvant chemotherapy has been associated with the absence of ERCC1 protein in patients with NSCLC.¹³ High tumoral expression of RRM1 (ribonucleotide reductase subunit M1) has been shown to predict resistance of NSCLC to gemcitabine and platinum combination therapy.¹⁵ High RRM1 expression has also been correlated with favorable prognoses (disease-free and overall survival) in early-stage NSCLC, although a significant association between RRM1 expression and histologic subtype was not observed.¹⁶ As pharmacogenomic research continues, additional genes and proteins may be identified that are associated with efficacy and safety outcomes.

NSCLC: Histologic Subtypes

The main histologic subtypes of NSCLC, as defined by the World Health Organization (WHO), include adenocarcinoma, squamous cell (epidermoid) carcinoma, and (anaplastic) large cell carcinoma.¹⁷ The distribution of these histologic subtypes has changed worldwide in the last few decades, marked by a growing incidence of adenocarcinoma and a concurrent decline in the incidence of squamous cell carcinoma.¹⁸ The epidemiology, global distribution, and

causative factors associated with lung cancer have recently been reviewed in depth.^{18,19}

Squamous cell carcinoma has historically had the strongest association with smoking, although it is believed that design changes in cigarettes (lower tar, lower nicotine, and side vents) have changed the anatomic location and histologic subtype of lung cancer.^{20–23} Squamous cell carcinoma arises most frequently in the proximal bronchi and tends to remain localized. The histologic diagnosis of classic squamous cell carcinoma is generally straightforward, with relatively extensive areas of keratinization and an associated inflammatory component, especially in lesions undergoing cavitation. Less differentiated forms of squamous cell carcinoma, however, have no keratinization and are made of smaller undifferentiated cells. These cells may recapitulate basal cell layers of the squamous epithelium, and, indeed, basaloid variants of squamous cell carcinoma and large cell carcinoma have also been described.²⁴ Recently, these variants were reported to have a significantly worse prognosis compared with conventional squamous cell carcinoma.²⁴ Such tumors should be distinguished from small cell lung carcinoma, and immunohistochemistry for neuroendocrine markers or specific types of cytokeratins may be required.

Adenocarcinoma includes a morphologically heterogeneous group of tumors.^{25,26} It is also associated with smoking, although more and more adenocarcinomas occur in “never smokers” (generally in young women). Patients often present with metastatic disease before the development of symptoms. Most of these tumors are peripheral and are related to surface alveolar epithelium or to bronchial mucosal glands. Histologic examination reveals gland formation, papillary structures, or solid growth with mucin production. One subtype of adenocarcinoma, bronchioalveolar carcinoma (BAC), is a more slowly growing tumor with a different clinical presentation and a better prognosis compared with other adenocarcinomas.^{27,28} The histopathologic definition of BAC has undergone major changes in recent years, and some confusion exists regarding its diagnosis among different centers if the currently proposed strict diagnostic criteria are not correctly applied.^{29,30} The diagnosis of BAC is now restricted to tumors that have a lepidic growth along pulmonary septa in the absence of parenchymal invasion.^{29,30} All tumors originally diagnosed as BAC, therefore, need to be reassessed in any retrospective study since, in past decades, the term BAC has been applied to tumors with a remarkably different behavior. In this context, the number of pure BAC tumors is low and includes both mucinous and nonmucinous forms. Additionally, because many pulmonary adenocarcinomas have a combined pattern of growth, mixed areas of BAC and conventional acinar or papillary adenocarcinoma may coexist in the same tumor. Indeed, such tumors are so common that the WHO proposed the classification “mixed subtype” for these adenocarcinoma variants.¹⁷ The mixed subtype generally follows the behavior of conventional adenocarcinoma, with the possible exception of tumors with a minimal invasive component. One study has, in fact, shown that minimal parenchymal invasion does not significantly alter the excellent prognosis of predominantly BAC-type tumors.³¹

Large cell carcinoma is the least common of the three main subtypes of NSCLC. The criteria for its diagnosis are not well defined and vary widely. Modified histopathologic diagnostic criteria indicate that some tumors categorized as large cell carcinoma should be reclassified as undifferentiated adenocarcinoma or squamous cell carcinoma, but a lack of morphologic features often prevents an appropriate classification. This is especially true in advanced NSCLC, which comprises high-grade undifferentiated primary or metastatic tumors that are often inoperable. Thus, the histologic material for the diagnosis is limited to small biopsy samples (of primary or metastatic tumors), and the final tumor type classification may not be correct. Nevertheless, the latest WHO classification¹⁷ has dramatically changed the criteria for “anaplastic large cell carcinomas” by introducing a separate category of “sarcomatoid carcinoma” and including only very rare tumor subtypes (lymphoepithelial, clear cells, and rhabdoid variants). A notable exception to the lack of distinctive histologic criteria is the large cell neuroendocrine carcinoma, recognized by Travis and colleagues as a distinct tumor entity with neuroendocrine features and a clinical behavior similar to that of small cell lung cancer.³² Large cell neuroendocrine carcinoma should be identified using neuroendocrine markers and should be excluded from studies in NSCLC because of its different biologic and clinical properties.

The biologic features and treatment sensitivity of the rarer NSCLC subtypes, such as sarcomatoid tumors, are not as well defined. For the purpose of analysis, these rarer tumors are often grouped with unspecified and poorly differentiated NSCLC. Mixed carcinomas, which may contain all possible combinations of NSCLC subtypes (for example, adenosquamous), also exist. A mixed tumor that contains a small cell carcinoma component and a NSCLC component is considered a small cell lung cancer combined variant and, as such, should be excluded from NSCLC studies.

METHODS

Study Selection

This review focuses on histology in unresectable, stage III/IV NSCLC. Literature searches were conducted using the Scirus search engine, which contains over 450 million indexed scientific items. The sources indexed by Scirus include: Medline, ScienceDirect, BioMed Central, and Nature Publishing Group. Searches covered the interval from 1982 to 2007. Search terms included combinations of the following in both the title and text of the article: non-small cell lung cancer, advanced, metastatic, phase II, phase III, randomized/randomised, controlled trial, systematic review, meta-analysis, retrospective analysis, chemotherapy, predict/predictive, prognosis/prognostic, interaction, multivariate, univariate, Cox (proportional hazard model), histology/histologic/histotype, adenocarcinoma, and squamous cell carcinoma. Articles that were published in a language other than English and congress abstracts were excluded.

For this review, the methods and results section of each article were reviewed to identify any association of histology with efficacy variables. Reference lists from each article were

searched for additional relevant publications, as were reference lists from relevant review articles. A predefined template was used to collect information from each publication including study design, methods, population characteristics, and results. Compiled data were then reviewed, using each publication as the reference source. Any discrepancies/differences found were discussed and agreed upon.

Statistical Considerations

In the context of this review, histologic subtype is considered “prognostic” when it is associated with a clinical outcome (prognosis) independent of a given therapy. Histologic subtype is considered “predictive” when it is associated with, and predicts, the effectiveness of a particular treatment, i.e., when there is a significant treatment-by-histology interaction.^{33,34} The presence of a significant treatment-by-histology interaction indicates that the treatment effect (for example, the hazard ratio for survival) varies according to the histologic diagnosis. That is, the combination of the treatment and the histologic diagnosis affects the efficacy outcome (such as survival). For the exploratory purpose of this review, a 0.10 significance level was chosen, regardless of what level each publication used, to have a standard definition to apply across all studies. Studies that evaluated different doses of the same therapy (without a control arm) are not informative in ascertaining a predictive association and were not considered in this review.

In the tables presented in this review, the results for prognostic factors are reported using univariate analyses and/or multivariate analyses, and predictive factors are reported using multivariate analyses (as multivariate analyses are required to assess an interaction effect); these results were compiled from those reported in each publication. Although histology may have a prognostic and/or predictive association in each of the selected studies, in some cases, other factors (such as disease stage, gender, performance status, or prior therapy) may have been reported to have a greater impact than histology; however, these factors are not summarized in this review.

RESULTS

We identified 408 publications that contained the search terms. Of these 408 publications, we excluded those that had not tested for a prognostic or predictive role for histology. Also excluded were publications that examined this relationship but reported no statistically significant evidence. This review summarizes 32 publications that reported a statistically significant association between histology and one or more efficacy parameters.

Of the publications reporting an association between histology and treatment outcome, 14 summarized studies of tyrosine kinase inhibitors. These papers were examined separately. The remaining 18 publications on cytotoxic chemotherapy were divided into two groups: those that examined the association of histology with efficacy variables in a single population, and those that examined this relationship comparing two or more populations or treatment arms. The two groups of chemotherapy publications are discussed in the next two sections and are summarized in Tables 1 and 2. The

TABLE 1. Single-Arm or Pooled Population Studies Showing NSCLC Histologic Subtype as a Possible Prognostic and/or Predictive Factor of Clinical Outcomes

Study (First Author, Date of Publication)	Treatment Arms ^a (n)	Observed Histology Subtypes (% of Each) ^b	Histology Results	Conclusions
Retrospective analyses of patient data pooled from multiple sites and/or multiple years				
Charloux, 1997 ³⁵	First-line CT and/or RT; (n = 141 unresected stage IIIA/B/IV)	For pts at all disease stages: Adeno (16) SQ (73) BAC (7) LC (4)	Median OS (mo): BAC: 12.5; SQ: 6.8; LC: 6.5; Adeno: 4.9 (<i>p</i> = 0.042). Multivariate analysis: relative risk for Adeno 1.47 (with SQ as reference) (<i>p</i> = 0.087); relative risk for BAC 0.47 (<i>p</i> = 0.056).	BAC associated with > OS; Adeno associated with < OS.
Martins, 1999 ³⁶	First-line CP-based CT, RT, surgical procedures (n = 616 stage IV)	Adeno-BAC (41) SQ (37) BAC (5) LC (4) Poorly-differentiated (13)	Multivariate analysis: OS HR for Adeno [0.76, <i>p</i> = 0.0080] and poorly differentiated [0.68, <i>p</i> = 0.0079] < SQ [1.00], indicating > OS. HR for LC [1.54, <i>p</i> = 0.0514] showed trend for < OS.	Adeno and poorly differentiated associated with > OS over SQ.
Itaya, 2007 ³⁷	First-line Cb + PTX (n = 98)	Adeno (76) Non-Adeno (24)	Multivariate analysis: OS (but not PFS) > for Adeno over Non-Adeno (2-yr survival rate 40% vs 15%, <i>p</i> = 0.0017).	Adeno associated with > OS.
Shinkai, 1992 ³⁸	First-line CT: CP + VIND, VIND + MIT, or CP + VIND + MIT (n = 192)	Adeno (70) SQ (18) LC (9) Adeno-SQ (3)	Univariate analysis: SQ favorable for tumor response (<i>p</i> = 0.017). Multivariate analysis: histology not associated with tumor response.	SQ associated with > tumor response (in univariate analysis).
Finkelstein, 1986 ³⁹	First-line CT: one of seven combination regimens (n = 893)	Adeno (43) SQ (36) LC (19) Other (2)	Multivariate analysis: Non-LC associated with > 1-yr survival.	Non-LC associated with > survival.
Analyses of patient data from clinical trials with either a single arm (phase II) or multiple pooled arms (phase III)				
Ceresoli, 2004 ⁴⁰	Second-line weekly PTX (n = 53)	Non-SQ (70) SQ (30)	> PFS for Non-SQ (<i>p</i> = 0.004). Multivariate analysis: Clinical benefit (CR+PR+SD) > for Non-SQ over SQ (<i>p</i> = 0.03, OR = 1.37).	Non-SQ associated with > clinical benefit and PFS.
LeCaer, 2007 ⁴¹	First-line weekly DTX (n = 50)	Adeno (40) SQ (48) Undifferentiated (12)	Univariate analysis: Histology (unstated cell type) associated with > OS. Multivariate analysis: no correlation between histology and OS.	Histology associated with > OS (in univariate analysis).
Weiss, 2007 ⁴²	Pooled arms: second-line DTX vs PEM (n = 571)	Adeno (52) SQ (30) Other (18)	Univariate analysis: OS > for Adeno over SQ or Other (9.1 vs 6.5 vs 7.8 mo, <i>p</i> = 0.004). Multivariate analysis: for the same comparison, <i>p</i> = 0.054.	Adeno associated with > OS.
Fukuoka, 1991 ⁴³	Pooled arms: CP + VIND vs CP + VIND + MIT vs CP + VIND + MIT + ETO (n = 203)	Adeno (48) SQ (43) LC (8)	Multivariate analysis: SQ associated with > tumor response (<i>p</i> = 0.0428), but not OS.	SQ associated with > tumor response.
Rapp, 1988 ⁴⁴	Pooled arms: Group A: VIND + CP vs CYP + DOX + CP vs BSC (n = 137)	Adeno (45) SQ (29) LC (22) Other (4)	Multivariate analysis: Histology associated with OS (<i>p</i> = 0.022), with SQ associated with < median OS than Adeno or LC.	SQ associated with < median OS.
Saynak, 2005 ⁴⁵	Pooled arms: CP + ETO with sequential or concurrent RT (n = 132)	Adeno (25) SQ (73) LC (2)	Univariate and multivariate analysis: Non-SQ associated with < OS (<i>p</i> = 0.04).	Non-SQ associated with < OS.

^a Data reported for stage IIIB/IV patients unless otherwise noted. Some cytotoxic chemotherapeutic agents reported in this summary table are no longer considered active in advanced NSCLC.

^b For studies with two or more arms, the percent of patients with tumors of each histology subtype is reported as a rounded average of the arms.

Adeno = adenocarcinoma, BAC = bronchioalveolar carcinoma, BSC = best supportive care, Cb = carboplatin, CP = cisplatin, CR = complete response, CT = chemotherapy, CYP = cyclophosphamide, DOX = doxorubicin, DTX = docetaxel, ECOG = Eastern Cooperative Oncology Group, ETO = etoposide, HR = hazard ratio, LC = large cell carcinoma, mo = months, MIT = mitomycin, NCIC = National Cancer Institute of Canada, NS = not specified, OR = odds ratio, OS = overall survival, PEM = pemetrexed, PFS = progression-free survival, PR = partial response, pts = patients, PTX = paclitaxel, RT = radiotherapy, SD = stable disease, SQ = squamous cell carcinoma, VIND = vindesine, VNR = vinorelbine, vs = versus.

TABLE 2. Comparative Studies Identifying Histology as a Possible Predictor of Clinical Outcomes

Study (First Author, Date of Publication)	Treatment Arms ^a (n)	Observed Histology Subtypes (% of Each) ^b	Histology Results ^c	Conclusions
Analyses of data from trials of cytotoxic chemotherapeutic agents				
Ardizzone, 2007 ⁴⁶	First-line CP-containing regimen (n = 1489) vs Cb-containing regimen (n = 1479)	Non-SQ (~62) SQ (~38)	Non-SQ, Cb associated with > mortality (HR = 1.12, 95% CI: 1.01–1.23). SQ: Cb not associated with mortality (HR = 0.97, 95% CI: 0.85–1.10) (interaction p = 0.098). OR for nonresponse (Cb vs CP) 1.58 (95% CI: 1.27–1.97) for Non-SQ and 1.10 (95% CI: 0.85–1.43) for SQ (interaction p = 0.046).	In meta-analysis, Non-SQ predictive of < odds of tumor response and > risk of mortality for Cb-containing regimens.
Rudd, 2005 ⁴⁷	First-line GEM + Cb (n = 212) vs MIT + IFO + CP (“MIC”) (n = 210)	Adeno (35) SQ (41) Other (24)	“Other”: > OS for GEM + Cb over MIC. Adeno: trend favoring GEM + Cb. SQ: no difference between treatments (interaction p = 0.10).	“Other” histology, and possibly Adeno, may predict > OS to GEM + Cb.
Georgoulas, 2001 ⁴⁸	First-line CP + DTX (n = 205 evaluable) vs GEM + DTX (n = 201 evaluable)	Adeno (35) Non-Adeno (65)	Non-Adeno > response to CP + DTX (40.4%) than GEM + DTX (27.6%) (p = 0.028). Adeno > response to GEM + DTX (43.2%) than CP + DTX (23.2%) (p = 0.011). Significant treatment-by-histology interaction for response (p-value not reported).	Non-Adeno predictive of > tumor response to CP + DTX; Adeno predictive of > response to GEM + DTX.
Cellerino, 1991 ⁴⁹	First-line CYP + EPI + CP alternating with MTX + ETO + LOM (n = 62) vs BSC (n = 61)	Adeno (32) SQ (60) LC (8)	Non-SQ: > OS for CT over BSC (9.0 vs 3.9 mo, p = 0.041). SQ: same OS in both arms (6.0 vs 5.6 mo, p = 0.94). Formal test of treatment-by-histology interaction not presented.	Non-SQ may predict > OS for CT regimens.
Veronesi, 1988 ⁵⁰	First-line CYP + ADR + MTX + ProC (“CAMP”) (n = 62) vs CP + ETO (n = 71)	SQ (68) Adeno (28) LC (3) Unclassified (2)	SQ: > response to CP + ETO over CAMP (45 vs 22%). Adeno: similar response to both treatments. Mantel-Haenszel chi-square (adjusted for histology) p-value = 0.08.	SQ may predict > tumor response for CP + ETO.
Analyses of data from trials of induction chemotherapy				
Kim, 2002 ⁵¹	Induction CT (CP + ETO + VIN) then RT (n = 53) vs RT alone (n = 48) (All pts stage IIIA/B)	Adeno (18) SQ (74) Other (8)	Non-SQ: median OS > for induction CT over RT (14.0 vs 3.6 mo, p = 0.027). SQ: median OS same for both groups (11.6 mo vs 9.4 mo, p = 0.853). Formal test of treatment-by-histology interaction not presented.	Non-SQ may predict > OS for CP + ETO + VIN induction CT prior to RT.
Huang, 2007 ⁵²	Induction CT (platinum + taxane) then RT + CT (n = 127) vs RT + CT (n = 138) (All pts locally advanced)	SQ (34) Adeno (39) LC (4) Unclassified/Other (23)	Induction CT associated with > OS for Non-SQ (Adeno or LC) (5-yr rate 24% vs 8%, p = 0.0033), but not SQ (p = 0.29). Formal test of treatment-by-histology interaction not presented.	Non-SQ may predict > OS for platinum + taxane induction CT prior to RT.

^a Data reported for stage IIIB/IV patients unless otherwise noted. Some cytotoxic chemotherapeutic agents reported in this summary table are no longer considered active in advanced NSCLC.

^b The percent of patients with tumors of each histology subtype is reported as a rounded average of the arms. Due to rounding, percents may not total 100%.

^c For the purpose of this review, a 0.10 significance level was selected to consider a test of interaction significant, regardless of what level the publication used, in order to have a standard definition across studies.

Adeno = adenocarcinoma, ADR = adriamycin, BSC = best supportive care, Cb = carboplatin, CI = confidence interval, CP = cisplatin, CT = chemotherapy, CYP = cyclophosphamide, DTX = docetaxel, EPI = epirubicin, ETO = etoposide, GEM = gemcitabine, HR = hazard ratio, IFO = ifosfamide, LC = large cell carcinoma, LOM = lomustine, MIT = mitomycin, mo = months, MTX = methotrexate, OR = odds ratio, OS = overall survival, ProC = procarbazine, RT = radiotherapy, SQ = squamous cell carcinoma, VIN = vinblastine, vs = versus.

tyrosine kinase inhibitor publications are discussed in the last section of the Results and are summarized in Table 3.

Histology as a Potential Prognostic Factor in Single-Arm or Pooled Population Studies

Eleven studies in the last 25 years have associated histology with clinical outcome in late-stage NSCLC.^{35–45} As summarized in Table 1, these studies are of 3 types: (1) retrospective analyses of pooled patient data, often generated outside of a clinical trial setting (first section of Table 1); (2) phase II clinical trial data without a comparator arm; (3) phase III clinical trial data in which data from the arms are pooled. (The phase II and III clinical trial data are listed together in the second section of Table 1.) Although each of the studies assigned a prognostic role for histology, because of the design of the studies and/or the types of analyses, it is unknown if the association between histology and clinical outcome is independent of treatment (prognostic) or predictive of the efficacy of a particular treatment. Given this uncertainty, the following discussion of these studies and the summary in Table 1 use general terms to describe the association instead of the terms “prognostic” and “predictive.”

In each of the retrospective analyses listed in the first section of Table 1, histology was associated with clinical outcome in an unresectable, locally advanced or metastatic NSCLC patient population. Patients were either treated at a single institution or within a cooperative group of institutions. In the first five studies listed, patients were treated with standard palliative care (as defined at the time of the study),^{35,36} with a combination of a platinum plus other drug(s),^{37,38} or with one of seven combination cytotoxic chemotherapy regimens.³⁹ Despite the difference in treatments and the span of years covered by these studies, all of the studies except Shinkai³⁸ found that adenocarcinoma or a subtype of adenocarcinoma was associated with better clinical outcome. In the Finkelstein analysis, the better outcome was extended to all nonlarge cell subtypes³⁹; in the Charloux analysis, the better outcome was associated with the BAC subtype, but not with all other adenocarcinomas.³⁵

In addition to the retrospective analyses of pooled patient data, Table 1 summarizes six clinical trials that also identified an association between histology and clinical outcome.^{40–45} In a multivariate analysis, the Ceresoli study found that nonsquamous histology was associated with clinical benefit (CR + PR + SD) in patients receiving weekly second-line paclitaxel.⁴⁰ In a similar study in patients receiving weekly first-line docetaxel, histology was associated with clinical outcome in a univariate analysis, but the association was no longer observed in a multivariate analysis.⁴¹

The Weiss, Fukuoka, and Rapp studies were all multi-arm trials that combined treatment arms for the analysis of histology associated with clinical outcome (Table 1).^{42–44} In a retrospective analysis⁴² of the phase III clinical trial comparing pemetrexed versus docetaxel,⁵ adenocarcinoma was associated with improved survival. The patients participating in the Fukuoka study all received cisplatin; in addition, patients received vindesine on one arm, vindesine plus mitomycin on another arm, and vindesine, mitomycin, and etoposide on a third arm. In a multivariate analysis, squamous

cell carcinoma was associated with improved response.⁴³ In a trial by Shinkai, which reproduced two of the vindesine-containing treatment arms of the Fukuoka trial, squamous cell carcinoma was associated with tumor response in a univariate analysis; however, in a multivariate analysis, only the number of metastatic sites was a significant baseline factor affecting tumor response.³⁸

In the analysis by Rapp et al.,⁴⁴ squamous cell carcinoma was associated with shorter survival for a pooled group of two cisplatin-containing treatment arms and a best supportive care (BSC) arm (Table 1). Although one of the two treatment arms (cisplatin plus vindesine) duplicated one of the three arms in the Fukuoka study, the results differed in that the Rapp study concluded that squamous cell carcinoma was associated with shorter survival and the Fukuoka study concluded that squamous cell carcinoma was associated with improved response with no effect on survival. This difference underscores the difficulty of interpreting results obtained by combining multiple treatment arms.

Histology as a Potential Predictor of Clinical Outcome in Comparative Studies

Seven studies were identified that found, through comparative analysis, that histology may predict efficacy outcomes to specific chemotherapy regimens (Table 2).^{46–52} The studies summarized in Table 2 are limited to those that provided statistically significant evidence that histologic subtype may predict outcomes to conventional chemotherapeutics. Studies are included that did not report a formal test of treatment-by-histology interaction. For those studies for which a treatment-by-histology interaction was not reported but there appeared to be a differential treatment effect by histology, we concluded that there may be a predictive association; however, a formal assessment of a treatment-by-histology interaction would have been more informative.

The first part of Table 2 lists studies of cytotoxic chemotherapeutic agents, with four of these studies comparing two chemotherapy regimens,^{46–48,50} and the study by Cellerino et al.⁴⁹ comparing a combination therapy and BSC. Although the studies listed in Table 2 primarily examine data from two-arm clinical trials, the first entry is a meta-analysis of 9 randomized clinical trials ($n = 2968$) comparing cisplatin- versus carboplatin-containing regimens.⁴⁶ This meta-analysis found histology was predictive of a survival advantage, with carboplatin-containing regimens associated with lower odds of tumor response and a higher risk of mortality for patients with nonsquamous tumors. Statistically significant tests of treatment-by-histology interaction (interaction test p value = 0.098 for survival, $p = 0.046$ for response) confirmed the predictive effect of histology on survival and tumor response.

In a phase III study comparing two platinum-containing treatment arms, the same trend was not observed as in the Ardizzone meta-analysis. Rudd et al.⁴⁷ found that histology characterized as “Other” (that is, tumors that were neither adenocarcinoma nor squamous cell carcinoma), and possibly adenocarcinoma, may predict longer overall survival for carboplatin plus gemcitabine over cisplatin, mitomycin, plus ifosfamide (interaction test p value = 0.10).

TABLE 3. Clinical Studies Showing NSCLC Histologic Subtype as a Possible Prognostic and/or Predictive Factor of Efficacy Outcomes to EGFR Tyrosine Kinase Inhibitors

Study (First Author, Date of Publication)	Treatment Arms ^a (n)	Observed Histology Subtypes (% of Each) ^b	Histology Results	Conclusions
Chang, 2006 ⁵⁴	≥ First-line gefitinib (n = 438)	Adeno (75) SQ (16) LC (1) Other (8)	Univariate analysis: OS > in Adeno (p = 0.008). Multivariate analysis: tumor response > in Adeno (p = 0.008).	In retrospective analysis, Adeno associated with > tumor response.
Kaneda, 2004 ⁵⁵	First- to fourth-line gefitinib (n = 101)	Adeno (80) SQ (18) Other (2)	Univariate analysis: tumor response associated with Adeno (p = 0.0104). OR > 3 times higher for Adeno than Non-Adeno, not significant due to small number Non-Adeno pts.	In retrospective analysis, Adeno associated with > tumor response.
Fukuoka, 2003 ⁵⁶	Second- or third-line gefitinib, two doses (n = 210)	Adeno (66) SQ (20) LC (9) Other (5)	OR for responding 3.45 times > for Adeno than other histologies (p = 0.021).	Adeno associated with > tumor response.
Park, 2004 ⁵⁷	≥ Second-line gefitinib (n = 111) (stage IV)	Adeno (64) SQ (27) Other (9)	Multivariate analysis: Adeno associated with > tumor response (OR = 4.9, p = 0.006), disease control rate (OR = 4.1, p = 0.008), and > OS (p = 0.005) than Non-Adeno.	Adeno associated with > tumor response, disease control rate, and OS.
Jänne, 2004 ⁵⁸	Second-line (or higher) gefitinib (n = 195)	Adeno (46) NS NSCLC (26) BAC (10) SQ (8) LC (10)	Median OS 7.0 mo (95% CI: 4.4–9.6) for Adeno vs 3.0 mo (95% CI: 2.1–3.9) for Non-Adeno (p = 0.002).	Adeno associated with > OS.
Kim, 2005 ⁵⁹	≥ Second-line gefitinib for 94 pts, first-line for 4 pts (n = 98)	Adeno (42) BAC (6) SQ (41) NS NSCLC (11)	Multivariate analysis: Adeno associated with > tumor response than Non-Adeno (41% vs 9.8%, p = 0.001; OR = 4.30, p = 0.03). Median OS 299 days for Adeno vs 133 days for Non-Adeno (p = 0.009). Median TTP 385 days for Adeno vs 111 days for Non-Adeno (p = 0.002).	Adeno associated with > tumor response, TTP, and OS.
Lin, 2006 ⁶⁰	First-line gefitinib (n = 53)	Adeno (66) SQ (19) NS NSCLC (15)	Adeno associated with > tumor response (40.0 vs 16.7%, p = 0.07), > PFS (3.9 vs 1 mo, p < 0.0001), and > median OS (9.8 vs 7.3 mo; OR = 10.9, p = 0.004 in multivariate analysis) over Non-Adeno.	Adeno associated with > tumor response, PFS, and OS.
Yang, 2006 ⁶¹	First-line gefitinib (n = 196)	Adeno (73) SQ (15) Other (11)	Adeno associated with > tumor response (49% vs 25%, p = 0.0031), > disease stabilization (66 vs 44%, p = 0.006), and > OS (9.4 vs 4.3 mo, p = 0.0223) over Non-Adeno. OR = 2.84, p = 0.0039, in favor of Adeno.	Adeno associated with > tumor response and OS.
Cappuzzo, 2005 ⁶² (Cappuzzo, 2004 ⁶³ for additional study details)	Second-line gefitinib (n = 106)	Adeno (55) SQ (20) BAC (13) Undifferentiated (10) LC (2)	Mean difference of median OS for Adeno + BAC vs SQ + LC + Undifferentiated = 5 mo (log-rank p = 0.03).	Adeno + BAC associated with > OS.
Kris, 2003 ⁶⁴	≥ Third-line gefitinib, two doses, (n = 216)	Adeno (66) SQ (15) LC and Other (19)	Treatment arms pooled for analyses. Univariate analysis: Adeno associated with > tumor response (13% Adeno vs 4% for Non-Adeno, p = 0.046). Multivariate analysis: histology not significant.	Adeno associated with > tumor response (in univariate analysis).
Miller, 2004 ⁶⁵	First- to fourth-line gefitinib, (n = 139)	Adeno-non-BAC (60) BAC (17) Other (22)	Univariate analysis: BAC associated with > tumor response (38% BAC vs 14% Adeno-non-BAC, p < 0.001). Tumor response for Other was 0%. Multivariate analysis: OR: BAC 13.5, Adeno-non-BAC 5.2, Other 1.0, p = 0.01.	BAC associated with > tumor response over Adeno-non-BAC. Adeno-non-BAC associated with > tumor response over Other.

(Continued)

TABLE 3. (Continued)

Study (First Author, Date of Publication)	Treatment Arms ^a (n)	Observed Histology Subtypes (% of Each) ^b	Histology Results	Conclusions
Thatcher, 2005 ⁶⁶	Second- or third-line gefitinib (n = 1129) vs placebo (n = 563)	Adeno (45) SQ (34) LC (6) BAC (3) Mixed (2) Undifferentiated (10)	BAC combined with Adeno for analysis. Cox analysis: Adeno + BAC associated with > median OS in gefitinib over placebo (6.3 vs 5.4 mo, $p = 0.033$; log-rank $p = 0.089$). Non-Adeno not reported. Adeno + BAC (but not Non-Adeno) associated with > tumor response for gefitinib over placebo. Formal test of treatment-by-histology interaction not presented.	Adeno + BAC may predict > OS and tumor response following treatment with second- or third-line gefitinib.
Shepherd, 2005 ⁶⁷	Second- or third-line erlotinib (n = 488) vs placebo (n = 243)	Adeno (50) SQ (30) Other (20)	Univariate analysis of erlotinib treatment arm: Adeno associated with > response over Non-Adeno (13.9% vs 4.1%, $p < 0.001$); association remained in multivariate analysis ($p = 0.01$). Univariate analysis comparing survival for erlotinib vs placebo: HR = 0.7 ($p = 0.008$) for Adeno and HR = 0.8 ($p = 0.07$) for Non-Adeno. Multivariate treatment arm analysis: Adeno associated with > OS (HR = 0.8, $p = 0.004$). No significant treatment-by-histology interaction.	Adeno associated with > tumor response for erlotinib. Adeno associated with > OS in pts treated with placebo or erlotinib.
Johnson, 2005 ⁶⁸ (Further exploratory analysis of Shepherd, 2005 ⁶⁷)	Second- or third-line erlotinib (n = 488) vs placebo (n = 243)	Adeno (50) SQ (30) Other (20)	(Of note, Johnson used classification of Adeno, SQ, and Other, whereas Shepherd used Adeno vs Non-Adeno.) Univariate analysis: erlotinib > OS vs placebo in Adeno (HR = 0.71, 95% CI: 0.6–0.9) and SQ (HR = 0.67, 95% CI: 0.5–0.9), but not Other (HR = 1.04, 95% CI: 0.7–1.5). Formal test of treatment-by-histology interaction not presented.	In univariate analysis, Adeno and SQ may predict > OS for erlotinib.

^a Data reported for stage IIIB/IV patients unless otherwise noted.^b For studies with two or more arms, the percent of patients with tumors of each histology subtype is reported as a rounded average of the arms. Due to rounding, percents may not total 100%.
Adeno = adenocarcinoma, BAC = bronchioalveolar carcinoma, CI = confidence interval, HR = hazard ratio, LC = large cell carcinoma, mo = months, NS = not specified, OR = odds ratio, OS = overall survival, PFS = progression-free survival, pts = patients, SQ = squamous cell carcinoma, TTP = time to progression, vs = versus.

In a study by Georgoulas et al.⁴⁸ ($n = 406$), histology was predictive for 2 treatment regimens: cisplatin plus docetaxel and gemcitabine plus docetaxel (Table 2). Patients with adenocarcinoma exhibited significantly greater response to gemcitabine plus docetaxel, whereas patients with nonadenocarcinoma exhibited significantly greater response to cisplatin plus docetaxel, with a significant treatment-by-histology interaction test for tumor response (interaction test p value not reported). Although histology was predictive of tumor response, additional analyses determined that histology was not predictive of survival or progression-free survival.

A retrospective data analysis of the Cellierino trial found that nonsquamous carcinoma may predict longer survival for patients treated with the chemotherapy regimen versus BSC (Table 2).⁴⁹ In contrast, patients with squamous cell carcinoma had the same median overall survival in both treatment arms. This conclusion is tentative because of the lack of a formal treatment-by-histology interaction test and the small size of the trial. The results of the Veronesi study ($n = 133$) also suggested that histology may predict tumor response; the test for association between treatment and response, adjusted for histology, resulted in a p value of 0.08.⁵⁰ In this study, patients with squamous cell carcinoma had double the response rate to cisplatin plus etoposide compared with a four-drug combination regimen (44.7% versus 21.6%), whereas patients with adenocarcinoma had a comparable response to both treatments (23.5% versus 27.3%).

A separate section of Table 2 summarizes the possible predictive role of histology in two studies of induction therapy.^{51,52} In both of these studies, specific induction chemotherapy regimens before radiation preferentially extended survival for patients with nonsquamous histology. In contrast, patients with squamous cell carcinoma did not experience any survival benefit from induction therapy. A third study showed a similar trend, but did not reach statistical significance.⁵³ Although differential treatment effects were observed within the histology subtypes in these studies, a formal test of treatment-by-histology interaction, which could have confirmed histology as a predictive factor, was not reported. In a study by Saynak et al.⁴⁵ (Table 1), patients received similar chemotherapy with concomitant and sequential radiation. In this study, nonsquamous histology was associated with shorter survival; however, “predictiveness” could not be assessed because the treatment arms were pooled for the statistical analysis.

Histology as a Prognostic and/or Predictive Factor of Clinical Outcomes to Tyrosine Kinase Inhibitors

Consistent evidence of an association between histology and clinical outcome following treatment with EGFR tyrosine kinase inhibitors was found in the 14 publications of 13 studies summarized in Table 3.^{54–62,64–68} The first 11 studies showed that adenocarcinoma was associated with superior response rates, disease control rates, progression-free survival, and survival in patients with advanced NSCLC, but squamous cell carcinoma or a pooled group of all nonadenocarcinoma subtypes was not.^{54–62,64,65} In one study, the

BAC subtype was examined separately and was associated with a significantly higher tumor response rate than other adenocarcinoma subtypes.⁶⁵ In all 11 of these studies, the patients were treated with the tyrosine kinase inhibitor, gefitinib, suggesting that adenocarcinoma may predict better efficacy outcomes for this agent; however, since all 11 studies lacked a nongefitinib comparator arm, this predictive association cannot be assessed.

The study by Thatcher et al.⁶⁶ also reported that adenocarcinoma may predict improved efficacy outcomes in patients with advanced NSCLC treated with gefitinib (Table 3). The large ($n = 1692$) prospectively randomized trial compared gefitinib with placebo. Although the differences in survival between the gefitinib and placebo arms did not reach significance in the primary log-rank test in either the overall or adenocarcinoma population, a prospectively defined Cox regression analysis suggested significance in favor of gefitinib for both the overall ($p = 0.03$) and adenocarcinoma ($p = 0.033$) populations. Furthermore, an exploratory subgroup analysis showed a higher response rate for gefitinib versus placebo in patients with adenocarcinoma; patients with nonadenocarcinoma did not exhibit a higher response for gefitinib versus placebo. These analyses suggest that there is a differential treatment effect, stronger in the adenocarcinoma subtype than in the nonadenocarcinoma subtype; however, a formal test of treatment-by-histology interaction was not reported.⁶⁶

The last two publications listed in Table 3 are analyses of the phase III trial of another tyrosine kinase inhibitor, erlotinib, versus placebo in patients who failed previous therapy.^{67,68} In the original analysis, adenocarcinoma was associated with better response and survival in a pooled population of patients treated with placebo or erlotinib.⁶⁷ In an exploratory univariate analysis of the same study, Johnson et al.⁶⁸ showed that squamous carcinoma and adenocarcinoma may both predict superior survival in erlotinib-treated patients; however, a formal test of treatment-by-histology interaction was not reported. Of note, the analysis in the original publication categorized histology as adenocarcinoma versus nonadenocarcinoma, whereas the analysis by Johnson et al. used the categories of adenocarcinoma, squamous cell carcinoma, and “other.” The difference in how squamous cell carcinoma was grouped for the analyses may account for the slightly different results.

DISCUSSION

Prompted by recent data from three randomized trials that identified an association between histology and treatment outcomes in advanced NSCLC,^{4,6,7} we examined the literature of the last 25 years to determine if other studies had also observed this relationship. Historically, histology has not been clearly or consistently described in the literature as a prognostic or predictive variable in advanced NSCLC studies. As reflected in this review of the published literature, the data are inconsistent with some studies suggesting more favorable outcomes for patients with adenocarcinoma or nonsquamous histologies, and other studies suggesting more favorable outcomes for patients with squamous cell carci-

noma. Although substantial differences in study design and analyses make such specific conclusions regarding the prognostic and predictive role of histology difficult, some studies were identified in which histology was associated with clinical outcomes. We identified 11 single-arm or pooled population studies that identified an association between histology and clinical outcomes in late-stage NSCLC (Table 1).^{35–45} In each of the 11 studies, the design of the study and/or type of analyses did not allow us to clearly distinguish between a prognostic or predictive association. In some cases, histology may have had both a prognostic and predictive role. Seven additional comparative studies found that histologic subtype may predict the efficacy outcomes of specific conventional cytotoxic chemotherapies (Table 2),^{46–52} and 14 publications of 13 studies found histology was prognostic and/or predictive in patients treated with EGFR tyrosine kinase inhibitors (Table 3).^{54–62,64–68} Although the literature search undertaken for this review article was extensive, additional publications may exist that support or contradict an association between histology and efficacy outcomes.

The most consistent link between histology and treatment outcome was found for EGFR tyrosine kinase inhibitors (Table 3).^{54–62,64–68} Interestingly, the association between adenocarcinoma and improved efficacy in patients treated with EGFR inhibitors was not an anticipated result; rather, it was initially hypothesized that the better outcome would correlate with squamous cell carcinoma, in which EGFR is highly expressed.^{69,70} More recent data suggest that the efficacy of EGFR tyrosine kinase inhibitors may be linked to their ability to block aberrant EGFR pathway signaling associated with activating receptor mutations,^{71,72} or that it may correlate with increased EGFR gene copy number.⁶² Despite the lack of a definitive understanding of the mechanism of action of EGFR inhibitors, adenocarcinoma histology can be used to identify those patients more likely to benefit from EGFR tyrosine kinase inhibitors.

A limitation of this review, as with any review or meta-analysis, is publication bias. Publication bias occurs when negative results (negative histology results in our case), which are often not published, are excluded. Analyses of efficacy by histologic subtype may not be reported for several reasons: the histology data were not collected; analyses were not performed because the study was inadequately powered or because historical evidence suggested such analyses were not important; analyses were performed but results were negative (and/or inconsistent across other endpoints) and therefore not reported; or results of analyses were positive but not reported because it was unclear how to interpret the findings.

Although many trials do not report treatment-by-histology analyses, while reviewing the search results, publications were identified that examined this relationship and found no significant association. A number of these publications analyzed phase III clinical trial data.^{53,73–85} Several of these studies combined treatment arms for the analysis of histologic subtypes and efficacy outcomes, thus precluding identification of a predictive relationship. Additionally, many of these studies were relatively small (100–200 patients per treatment

arm), and did not have adequate statistical power to detect an interaction. Several phase II studies were identified that also reported no association between histology and efficacy outcomes. Given the limited sample size of phase II studies, these findings were not unexpected.

There are multiple reasons why some studies that examined histology as a prognostic/predictive factor did not observe an association. The most obvious reason is that the study might have correctly concluded that histology is neither significantly prognostic nor predictive of efficacy outcomes. This might especially be true for studies investigating a predictive role for cytotoxic chemotherapy agents that affect structures (for example, enzymes or receptors) equally common to all cells.

Another reason that histology may fail to be identified as a prognostic or predictive factor is that the study design, study population, and/or statistical analyses are inadequate for this purpose. For example, meta-analyses, which combine results from different trials with various treatments and patient demographics, can obscure differences among various subgroups of patients.⁸⁴ Analyses that combine treatment arms can result in different conclusions than those that examine the arms separately; by definition, the former may only examine for prognostic significance whereas the latter may identify predictive effects. As an example, following the pivotal phase III study of second-line pemetrexed compared with docetaxel,⁵ a retrospective analysis by Weiss et al.⁴² pooled the treatment arms and reported a prognostic role for histology. However, in a second set of retrospective analyses recently presented for the same study, the treatment arms were evaluated separately and histology was predictive of a differential treatment effect for pemetrexed.⁴ Study features may also obscure specific histology findings. For example, subtle differences in the proportion of patients with specific baseline characteristics, such as performance status, disease stage, or adenocarcinoma, squamous cell carcinoma, or large cell carcinoma, may skew a study's results, making it easier or more difficult to find histology effects.

In many studies, inadequate study design equates with insufficient sample size. The requirement for sample size when testing for interaction (with time to failure as the outcome) is approximately four times larger than that needed to detect a main effect of the same magnitude, even with some favorable assumptions.^{86,87} Of note, the studies that observed a predictive effect, with a significant treatment-by-histology interaction, were often relatively large trials.^{46–48} An insufficient sample size may also lead to the combination of histologic subtypes, which could obscure a significant effect in a relatively uncommon histologic subtype (for example, large cell carcinoma).

Even within a morphologically defined histologic subtype, such as adenocarcinoma, multiple subtypes can exist, each associated with a different prognosis and/or responsiveness to a particular drug. When these subtypes are analyzed together, the better prognosis or responsiveness of some subtypes may be diluted by the poorer prognosis and nonresponsiveness of others, thus obscuring the advantage of specific histologic subtypes. For example, Charloux et al. and

Miller et al. concluded that BAC is associated with improved outcome,^{35,65} yet most studies combine this subtype (which often is present in relatively few patients) with other adenocarcinomas, thereby possibly masking its responsiveness. Similarly, because generally less than 10% of patients have large cell carcinoma (as shown in Tables 1 through 3), the grouping of large cell carcinoma with either adenocarcinoma or squamous cell carcinoma may also affect conclusions regarding the prognostic role of histology.

Additional evidence for differential responsiveness within the adenocarcinoma subtype has been shown in gene profiling studies.^{88–90} In these studies, the investigators found that each morphologic classification exhibited a unique pattern of gene expression, but there was heterogeneity in the gene profile within the adenocarcinoma classification such that the tumors could be subdivided further into multiple subtypes (three in one study, four in the other). Each subtype expressed unique genes characteristic of specific morphologic variants such as neuroendocrine or type II pneumocytes. Interestingly, both investigators showed that the median survival of the adenocarcinoma subtypes differed. Likewise, gene profiling has shown heterogeneity within the squamous cell carcinoma subtype, with two subclasses detected that differed in survival.^{91,92} These results provide additional evidence that further subdivision (defined either by histology or pharmacogenomic markers) of the adenocarcinoma and squamous cell carcinoma subtypes may be necessary to ascertain differences in prognosis and/or drug responsiveness.

A significant number of misclassified tumors within a study may also obscure the prognostic or predictive role for histology. As the definition of NSCLC histologic subtypes has evolved in recent years, the new criteria may not be equally applied across clinical institutions, potentially yielding misclassified tumors. Studies in which inter-reviewer variability was examined suggest that this is higher in the case of undifferentiated tumors (such as large cell carcinoma).^{93,94} Also, the tumor sampling method can affect the accuracy of the results. Diagnoses based on cytology specimens are limited because of a lack of information on tumor architecture. In a study comparing diagnoses obtained from bronchial biopsy samples versus thoracotomy samples, there was good agreement ($\kappa = 0.70$) between the 2 sample methods, with conflicting results most frequently seen in bronchial biopsy specimens that had necrotic sections and an absence of differentiation.⁹⁴ Furthermore, diagnoses made from bronchial biopsies or limited surgical sections may also lead to inaccurate results because of tumor heterogeneity. In a study by Roggli et al.,⁹⁵ major tumor heterogeneity was observed in 45% of the carcinomas studied.

Conclusions and Recommendations

This review examined the impact of NSCLC histology on clinical outcomes. Given the development of targeted therapies and the refinement of histologic classifications, more studies should include an analysis of histologic subtypes and their association with efficacy outcomes. The need for these analyses is even greater given that the incidence of adenocarcinoma has markedly increased, thus requiring treatment options that are particularly effective in these tumors.

For some treatments, tumor histology, in addition to a growing panel of biomarkers, has the potential of delineating which patients will benefit, thus allowing patients to avoid unnecessary exposure and the associated risk of toxic side effects. Additionally, since one connection between histologic subtype and toxicity has been identified recently (i.e., patients with squamous cell tumors are at risk for hemorrhagic complications when treated with bevacizumab),⁹⁶ future studies should also include an analysis of the association between histologic subtypes and clinically relevant toxicities.

As our reliance on histologic subtype for determining optimal treatment increases, so does the importance of a definitive histologic diagnosis. Future studies should consider a central pathology review or another related method for verifying histologic diagnosis. Furthermore, given the multiple distinct histologic subtypes within adenocarcinoma, BAC and any other subtype with distinct pathologic features should be separately analyzed when possible. Additionally, large cell neuroendocrine carcinomas are more appropriately analyzed with small cell lung cancer rather than in the large cell carcinoma/NSCLC category.

As new trials in NSCLC are designed, consideration should be given to the randomization process. When possible, whether by minimization or stratification, randomization should consider factors for histology in addition to the well-known prognostic factors of gender, disease stage, performance status, and smoking history. Most of the studies presented in this review demonstrated associations between these well-known prognostic factors (in addition to histologic factors) and efficacy outcomes.

To assess the true predictive association between treatment and histology, inclusion of a formal treatment-by-histology interaction test is recommended.^{33,34} Testing the hypothesis of no treatment effect within different histologic subtypes does not determine if treatment effect varies by histology; it only determines if a treatment effect exists among various histologic subtypes.³³ In addition, such analyses of treatment effect within histologic subtypes ignore the variability between the subtypes.³³ These issues are properly addressed by assessing treatment-by-histology interaction in multivariate analyses. Tests of interaction can be performed using standard statistical software (such as SAS), and usually require a statistician to define the interaction term and statistical model correctly. In addition to reporting tests of interaction, the reporting of treatment effects (such as medians and/or hazard ratios with 95% confidence intervals) in each major histologic subtype (adenocarcinoma, squamous cell carcinoma, large cell carcinoma, and all other subtypes), when feasible, is encouraged. This illustrates the survival pattern across histologic subtypes (i.e., determines which subtypes have longer or shorter survival) and the relative effects of treatment within histologic subtypes.

To assess treatment-by-histology interactions, large studies are needed, often as much as four times larger, to detect an interaction compared with a main treatment effect.^{86,87} Most studies lack adequate power to detect an interaction, if one exists. Recognizing that it is often not practical to design trials to assess formal treatment-by-histol-

ogy interactions, researchers should continue to prespecify or retrospectively assess the effects of baseline patient and disease factors on efficacy and safety (including a formal assessment of interactions with treatment). As we progress toward targeted-patient care, such analyses will help identify patients who are candidates for specific therapies.

The need for improved efficacy outcomes drives the search and refinement of active agents in NSCLC. Tumor histology promises to be another area in which drug efficacy may be improved. Given the introduction of more targeted agents and a refinement in histologic classifications, the examination of tumor histology and its association with efficacy outcomes may aid in maximizing survival in patients with NSCLC.

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